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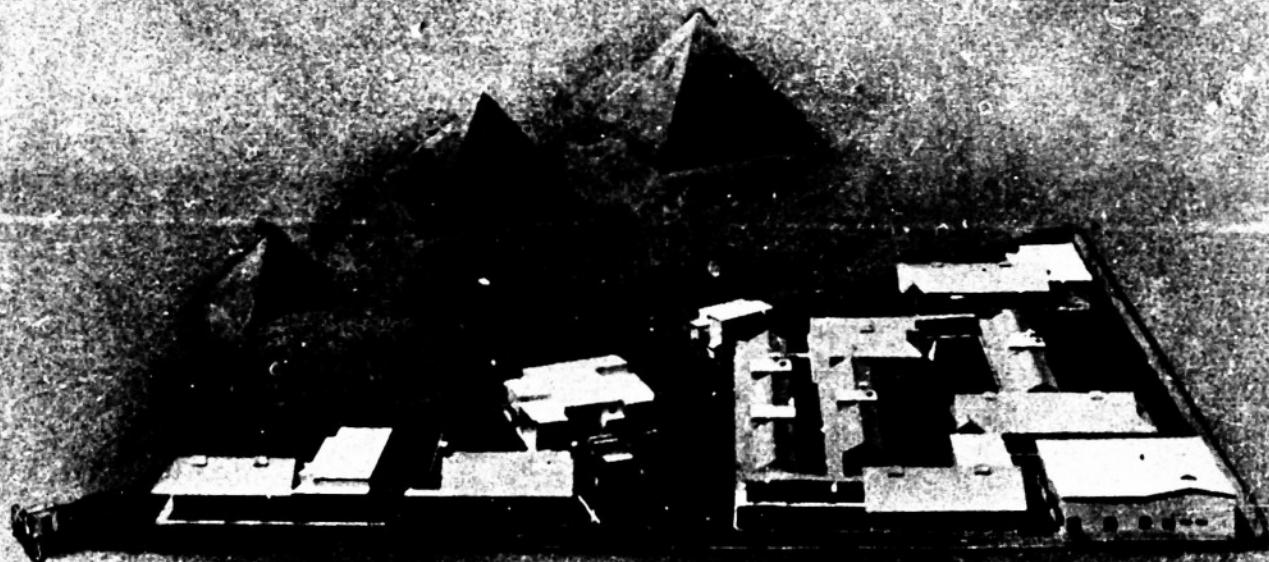
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CAIRO, EGYPT



THE EXPERIMENTAL TRANSMISSION OF A COXSACKIE-LIKE VIRUS  
BY MOSQUITOES

Herbert S. Hurlbut

With the technical assistance of E. V. Spangler  
U.S. Naval Medical Research Unit No. 3, Cairo, Egypt

Submitted to the Journal of the Royal Egyptian Medical  
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SUMMARY AND CONCLUSIONS

1. A Coxsackie-like virus originally isolated from mosquitoes was transmitted to suckling mice by the bites of experimentally infected mosquitoes of the species Culex pipiens L. and Culex univittatus Theobald.
2. C. pipiens transmitted the virus after feeding upon blood having titers of 7.9-8.8 followed by extrinsic incubation for 7-19 days. C. univittatus transmitted the virus under essentially the same conditions.
3. Propagation of the virus in C. pipiens was demonstrated by parenteral introduction and serial passage. After 5 serial passages with cumulative dilutions totaling  $10^{-9.2}$  virus recovery exceeded the original concentration and mosquitoes of the fifth passage transmitted the virus to suckling mice on which they fed.

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BY MOSQUITOES<sup>1</sup>

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These experiments deal with one of several strains of a Coxsackie-like virus isolated from mosquitoes in Egypt (1). The strain in question (Ar-339) was obtained from a pool of Culex pipiens L. and Culex univittatus Theobald.

Materials and Methods

The specimens of C. pipiens which were used in the transmission experiments were from a laboratory colony established in January, 1952 with larvae collected near Cairo, Egypt. The C. univittatus were reared from eggs deposited in the laboratory by field collected females. The mice were from a Swiss albino strain maintained at NAMRU-3.

The concentration of virus is expressed as the negative logarithm of the LD<sub>50</sub> dilution calculated according to the method of Reed and Muench. Five one-day old mice were inoculated intraperitoneally with 0.03 cc. of each serial decimal dilution of the virus. The results are based on the number of deaths occurring from 2-15 days after inoculation.

Parenteral infection of the mosquitoes was accomplished by a puncture technique whereby a minute steel needle wet with the virus suspension was thrust through the body wall of the thorax (2). Carbon dioxide was used to anesthetize the mosquitoes. The infected specimens were incubated at 28-32°C. and about 70 percent relative humidity. For estimates of virus concentration the whole bodies were triturated in a mortar and suspended in a ten per cent solution of inactivated normal rabbit serum containing 2000 units of penicillin and 3 mg. of streptomycin per cc.

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<sup>1</sup> The study was conducted under the auspices of the Ministry of Public Health of the Egyptian Government, and the U.S. Naval Medical Research Unit No. 3.

The opinions or assertions contained herein are the private ones of the author and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

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### Transmission Experiments

C. pipiens was infected initially by means of the puncture technique. The mosquitoes so infected were permitted to bite suckling mice after extrinsic incubation for one to two weeks. A viremia developed in the mice, which after 24 hours ranged in titer from 7.5 to 9.0. Uninfected mosquitoes were permitted to engorge on these mice and after incubation periods of 6-19 days the mosquitoes were allowed to bite normal suckling mice. These mice usually became ill on the second day and died on the second or third day. The data are summarized in Table I. C. pipiens transmitted the virus after feeding upon blood having titers of 7.9-8.6 and after extrinsic incubation periods of 7-19 days. C. univittatus given blood having titers of 7.5 and 9.0 transmitted the virus after incubation periods of 9-17 days.

Propagation of the virus in C. pipiens was demonstrated by parenteral introduction and serial passage. The data are presented in table 2. After 5 serial passages with cumulative dilutions equaling  $10^{-9.2}$  the triturated whole bodies of the fifth passage mosquitoes gave an  $ID_{50}$  of 6.9 when titrated in one-day old mice. The mosquitoes of this passage also transmitted the virus to suckling mice upon which they fed.

References

1. Taylor, R. M. and Hurlbut, H. S. The isolation of Coxsackie-like viruses from mosquitoes.
2. Hurlbut, H. S. The propagation of Japanese encephalitis virus in the mosquito by parenteral introduction and serial passage. Am. J. Trop. Med., 31:448-451, 1951.

TABLE 1

Transmission of Coxsackie-like virus (strain Ar339) to suckling mice by the bites of the mosquitoes, Culex pipiens L. and Culex univittatus Theobald

| Mosquito              | Infecting Mouse* |             |                |                                 | Extrinsic<br>Incubation<br>Days 28-32°C | Normal Mice Bitten |                  |                      |
|-----------------------|------------------|-------------|----------------|---------------------------------|---|--------------------|------------------|----------------------|
|                       | No.              | Age<br>Days | Incub.<br>Days | Blood<br>Titer LD <sub>50</sub> |   | Age<br>Days        | Number<br>Bitten | Number<br>Infected** |
| <u>C. pipiens</u>     | 1                | 4           | 2              | 7.9                             | 6-17                                    | 1-5                | 6                | 1                    |
|                       | 2                | 5           | 1              | 8.5                             | 12,18                                   | 2,3                | 2                | 2                    |
|                       | 3                | 6           | 1              | 8.2                             | 7-15                                    | 1-4                | 4                | 4                    |
|                       | 4                | 5           | 1              | 8.7                             | 9-19                                    | 1-5                | 4                | 4                    |
|                       | 5                | 5           | 1              | 8.3                             | 7-13                                    | 3-5                | 4                | 4                    |
|                       | 6                | 6           | 1              | 8.8                             | 12-16                                   | 4-5                | 3                | 2                    |
| <u>C. univittatus</u> | 1                | 5           | 2              | 7.5                             | 11-17                                   | 1-4                | 5                | 5                    |
|                       | 2                | 5           | 1              | >9.0                            | 9-14                                    | 1-4                | 3                | 3                    |

\* Infected by the bite of infected mosquitoes

\*\* Confirmed by bacteria free passage.

TABLE 2

Propagation of Coxsackie-like virus (strain Ar339) in Culex pipiens  
L. by parenteral introduction and serial passage.

| Passage No. | Log Dilution of Inoculum | Days of Incubation<br>28-32°C | LD <sub>50</sub> |
|-------------|--------------------------|-------------------------------|------------------|
| 1           | 1.3*                     | 15                            | 6.0              |
| 2           | 2.3                      | 5                             | >5.8             |
| 3           | 2.3                      | 4                             | 6.8              |
| 4           | 2.3                      | 4                             | 7.3              |
| 5           | 2.3                      | 3                             | 6.9              |

\* Mouse brain having an LD<sub>50</sub> of 7.5